



UNITED STATES ENVIRONMENTAL PROTECTION
AGENCY

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

NO CLAIMS OF CONFIDENTIAL BUSINESS INFORMATION

Review of Supplemental Information to OX5034 *Aedes aegypti*: Description of Starting
Materials, Production and Formulation Process, MRID 511309-01, April 23, 2020

Primary Reviewer: Chris A. Wozniak, Ph.D., Biotechnology Special
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Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist
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DATA EVALUATION RECORD

REQUIREMENT: EPA OCSPP

TEST MATERIAL: *Aedes aegypti* OX5034

SYNONYMS: OX5034

SPONSOR: Oxitec Ltd, 71, Milton Park, Abingdon, Oxfordshire, OX14 4RX
United Kingdom

AUTHOR: Oxitec Ltd.

TEST SITE: Milton Park, Abingdon, Oxfordshire, UK.

COMPLIANCE: Good Laboratory Practice Standards, 40 CFR Part 160, are not applicable to
this report.

I. BACKGROUND

Oxitec Ltd., (Oxitec) requests an Experimental Use Permit (EUP) under FIFRA Section 5 for a new end-use product containing the new active ingredient tetracycline-repressible transactivator protein variant (tTAV-OX5034) protein, the new inert ingredient DsRed2-OX5034 protein, and the genetic material (vector pOX5034) necessary for their production in OX5034 *Aedes aegypti* (Yellow Fever mosquito). Oxitec requests this EUP to evaluate whether the product is efficacious in suppressing naturally-occurring *Aedes aegypti* populations under field conditions.

OX5034 is described as a species-specific female larvicide, or “male-selecting” larvicide, that results in all-male progeny in the absence of tetracycline in the larval diet. With continued field releases of OX5034 homozygous males, the *Ae. aegypti* population is expected to progressively decline due to the reduced number of females emerging in the area. The same male-selecting trait is also harnessed in the manufacture of OX5034 to remove transgenic females from the mosquito population prior to the field releases, ensuring female mosquitoes are not released.

The self-limiting trait is made female-specific by inclusion of a splicing module, derived from the *Ae. aegypti Aeadsx (doublesex)* gene, that is naturally alternatively spliced between males and females as part of the sexual differentiation pathway (Salvemini et al., 2011). The alternative splicing has been genetically linked to the tetracycline-off (tet-off) system. The tet-off system activates expression of the tetracycline-repressible transactivator protein variant (tTAV) in females in the absence of a tetracycline analogue. All females carrying a copy of the transgene die during the larval stage due to the accumulation of tTAV protein produced by a positive feedback loop (Gossen & Bujard, 1992; Gong et al., 2005). In the absence of tetracycline analogues, females die whether they are homozygous or hemizygous for the OX5034 rDNA trait. If a suitable tetracycline analogue is added to the larval rearing medium in adequate amounts, tTAV expression is repressed, allowing for normal development of females to adulthood.

For suppression of *Ae. aegypti* populations, male OX5034 *Aedes aegypti* homozygous for the self-limiting gene are released into the environment to mate with wild type *Ae. aegypti* females; their offspring inherit a single copy (so are termed hemizygous) of the self-limiting gene. The self-limiting gene kills only female offspring (even those carrying only one copy of the self-limiting gene), which die at early larval stages of development. Hemizygous males will survive to pass the OX5034 genes on in further generations. Male mosquitoes do not bite humans or other animals.

DsRed2, a red fluorescent marker protein derived from *Discosoma* spp., is present in the OX5034 construct and aids in identification of OX5034 under laboratory conditions at the larval, pupal and adult stages. The red fluorescent phenotype can be detected following excitation with ultraviolet light and will be present whether the larva or adult is homozygous or hemizygous for the introduced trait.

OX5034 *Ae. aegypti* is derived through the transformation of a Latin type wild mosquito from Chiapas, Mexico by injection of pOX5034 along with a *piggyBac* transposase mRNA which does not persist in the final product. The Latin strain has been maintained in Oxitec labs since 2006.

The applicant requests a 24-month EUP for a cumulative annual test area of 6,600 acres. The area is divided into multiple test and control plots within Monroe, Co., Florida and Harris, Co., Texas. Under the EUP, Oxitec is planning to test the efficacy of the product by deploying adult male mosquitoes homozygous for the OX5034 trait.

II. STUDY PURPOSE

Biochemicals OCSPP Guideline 880.1200 Description of Starting Materials, Production, and Formulation Process

MRID 50889424, OX5034 *Aedes aegypti*: Description of Starting Materials, Production and Formulation Process, was previously reviewed, however, the Agency required clarification and supplemental information regarding the production process for *Ae. aegypti* OX5034.

EPA Request for Supplemental Information

EPA requested information regarding OX5034 production activities planned for Oxitec's facilities in the UK, in Monroe County, FL, and in Harris County, TX. Specifically, EPA requested information on which locations would carry out:

- Egg production, involving blood feeding of female mosquitoes, and use of tetracycline-class antibiotics.
- Adult male OX5034 rearing from eggs.

Oxitec OX5034 Production Facilities

UK Oxitec production facilities are located at: 71 and 37C Innovation Drive, Milton Park, Abingdon, Oxfordshire, United Kingdom, OX14 4RQ. Oxitec's UK facilities are a registered Pesticide Producing Establishment (Establishment No. 93167-GBR-1).

Oxitec has a dedicated biological containment level 2 facility used for production of insects in the United Kingdom (UK). The facility is licensed by the UK Health and Safety Executive (HSE) for the rearing of genetically modified (GM) organisms under contained use, as provided for by the UK Genetically Modified Organisms (Contained Use) Regulations (HSE, 20141).

OX5034 *Aedes aegypti* production is segregated from Oxitec's other *Aedes* rearing facilities. The production area is in a separate containment suite from other *Aedes* strains. The containment suite has a separate changing room and the insectaries are equipped with meshed air conditioning vents and floor drains. OX5034 *Aedes aegypti* are kept in at least three levels of containment from non-containment and other containment areas (i.e., the primary container, the insectary, and the changing room). Equipment used in the insectary is dedicated or is decontaminated by freezing for more than 12 hours at $\leq -15^{\circ}\text{C}$ before being brought into the containment suite. Staff working on OX5034 *Aedes aegypti* production are dedicated (i.e., Staff members who have entered other *Aedes* rearing areas on the same day are not permitted to access the OX5034 *Aedes aegypti* production suite).

All production of OX5034 eggs will take place in the UK at the abovementioned facility. These processes have been described fully in (Oxitec Ltd; MRID 50889424, 2019) and previously reviewed. These processes involve rearing female OX5034 in a dedicated insectary to produce eggs, which includes the use of blood feeding to enable females to lay eggs, and tetracycline-class antibiotic usage (specifically, doxycycline) in larval rearing to enable female OX5034 survival to adulthood. Eggs will be shipped to facilities in the USA for adult rearing and field deployment of eggs, as described in the Field Trial Protocol (Section G) of this EUP application.

Production facility in Florida

As described in the Field Trial Protocol (Section G of this EUP application), OX5034 will be deployed in Monroe County, FL and Harris County, TX.

Production facilities in Monroe County, FL will be used for adult rearing of OX5034 male mosquitoes for release, as described in (Oxitec Ltd; MRID 50889424, 2019; previously reviewed). Additionally, components of the Mosquito Rearing Box (e.g. larval diet, etc.) may be formulated from raw materials in this facility, and Mosquito Rearing Boxes assembled for deployment, as described in (Oxitec Ltd; MRID 50889424, 2019) and the associated SOPs (Oxitec Ltd; MRID 50889427; previously reviewed).

No egg production (involving blood feeding or tetracycline usage) will take place in this facility, and all OX5034 eggs used will be shipped from Oxitec's UK facilities. No other third-party mosquito strains will be reared in Oxitec's field trial laboratories in the USA.

Production facilities will be located at:

Florida Keys Mosquito Control District, 503 107th Street Gulf, Marathon, FL, 33050.

Production facility in Texas

Production facilities in Harris County, TX will be used for adult rearing of OX5034 male mosquitoes for release, as described in (Oxitec Ltd; MRID 50889424, 2019; previously reviewed). Additionally, components of the Mosquito Rearing Box (e.g. larval diet, etc.) may be formulated from raw materials in this facility, and Mosquito Rearing Boxes assembled for deployment, as described in (Oxitec Ltd; MRID 50889424, 2019) and the associated SOPs (Oxitec Ltd; MRID 50889427). No egg production (involving blood feeding or tetracycline usage) will take place in this facility, and all OX5034 eggs used will be shipped from Oxitec's UK facilities. No other third-party mosquito strains will be reared in Oxitec's field trial laboratories in the USA.

Production facilities will be located at:

Mosquito and Vector Control Division, 3330 Old Spanish Trail Bldg. D, Houston, TX, 77021.

Reviewer's Comments:

Oxitec has adequately clarified the production of eggs and adult OX5034 male mosquitoes, as well as construction of rearing boxes for mosquito release at their UK, Florida and Texas facilities. Key to this analysis is the indication that there will be no egg production or blood

feeding of adult female mosquitoes or use of tetracycline-class antibiotics taking place at the Florida or Texas facilities. Eggs will be shipped from the UK facility for deployment in the U.S. Further, the description of the dedicated containment suite at the UK facility, as used for production of OX5034 eggs, and the restriction of personnel who have visited other production suites from entering this dedicated insectary address production aspects of interest to the Agency.

CLASSIFICATION: ACCEPTABLE

References

Oxitec Ltd, MRID 50889424. 2019. *OX5034 Aedes aegypti: Description of Starting Materials, Production and Formulation Process.*

Oxitec Ltd, MRID 50889427. Standard Operating Procedures for Production of Aedes aegypti, OX5034. 2019.